Outline of the proposed work for the degree of doctor of philosophy in pharmaceutical sciences

Submitted To

SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY, NANDED.

Topic

"DESIGN AND EVALUATION OF GUT FLORA TARGETING PROPHYLAXIS FOR CHRONIC DISEASE"

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(2017-2018)

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FOR REGISTRATION OF SUBJECTS FOR

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4	**Date of admission to the Course:**	
5	**Title of the topic: "DESIGN AND EVALUATION OF GUT FLORA TARGETING PROPHYLAXIS FOR CHRONIC DISEASE"**	
6	**Brief resume of the intended work:** 1. **Introduction** : Enclosure –I **2. Review of Literature** : Enclosure -II **3. Need for the study** : Enclosure -III **4. Objectives of the study** : Enclosure -IV	
7	**Material and methods:** 1. **Source of the data** : Enclosure -V **2. Methods of the collection of the data** : Enclosure -VI **3. Does the study require any investigations or interventions to be conducted on patients other human or animals? If so, please describe briefly.** YES: (mice and rats)	
8	**List of References**	**ENCLOUSUR-VII**

9	**Signature of the candidate:**	**(WADULKAR RAGHUNATH D.)**
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ENCLOSURE-I

1.INTRODUCTION:

Chronic diseases are the leading causes of death and disability worldwide. Disease rates from these conditions are accelerating globally, advancing across every region and pervading all socioeconomic classes. The World Health Report 2002: Reducing risks, promoting healthy life, indicates that the mortality, morbidity and disability attributed to the major chronic diseases currently account for almost 60% of all deaths and 43% of the global burden of disease. By 2020 their contribution is expected to rise to 73% of all deaths and 60% of the global burden of disease. Moreover, 79% of the deaths attributed to these diseases occur in the developing countries. Four of the most prominent chronic diseases cardiovascular diseases (CVD), cancer, chronic obstructive pulmonary disease and type 2 diabetes are linked by common and preventable biological risk factors, notably high blood pressure, high blood cholesterol and overweight, and by related major behavioral risk factors: unhealthy diet, physical inactivity and tobacco use. Action to prevent these major chronic diseases should focus on controlling these and other key risk factors in a well-integrated manner.

Centers for Disease Control and Prevention (CDC) further states that 7 out of ten deaths each year are from chronic diseases, with heart disease, cancer and stroke accounting for more than 50 percent of all deaths each year. A majority of these diseases have numerous causes, most preventable; lack of physical activity, poor nutrition, tobacco use and excessive alcohol consumption are the most common culprits.

More than 100 trillion symbiotic microorganisms live on and within human beings and play an important role in human health and disease. The human microbiota, especially the gut microbiota, has even been considered to be an "essential organ" [1], carrying approximately 150 times more genes than are found in the entire human genome [2]. Important advances have shown that the gut microbiota is involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity [3–6].

In recent decades, a tremendous amount of evidence has strongly suggested a crucial role of the human microbiota in human health and disease [7, 8–9] via several mechanisms. The human microbiome can be considered as an important origin of resources for genetic diversity, a modifier of disease, an essential component of immunity, and a functional entity that influences metabolism and modulates drug interactions. On one hand, there are many potential probiotics or beneficial bacteria that may prevent or treat certain diseases, although most of them cannot be cultivated at

present [10]. For example, some of these gut microbes belong to genera that contain many probiotics such as *Lactobacillus* and *Bifidobacterium.* Some are novel potentially beneficial bacteria, such as *Faecalibacterium prausnitzii* is one of the most abundant and important commensal bacterium of the human gut microbiota for treating IBD and irritable bowel syndrome (IBS), and *Akkermansia muciniphila* is a species of human intestinal mucin degrading bacterium, *muciniphila* is believed to have anti-inflammatory effects in humans **.[11]**

On the other hand, as our second genome, the human microbiome must produce a large number of metabolites. Some isolated metabolites have important potential applications, although it still remains a great challenge to isolate and identify all the metabolites of the human microbiome**.** Enrichments of some microbes are noted as potential biomarkers in some research; however, these alterations are often observed in other research as well, and cannot be distinguished among different diseases. In contrast, clinical models based on tens of genes within a metagenome analysis perform better in diagnostics and predicting diseases. In addition, we found that the *Bifidobacterium* /*Enterobacteriaceae* (B/E) ratio indicates the microbial colonization resistance of the bowel, and that this ratio is considered to be an indicator of human microbiome health.[13, 14]. The prevention and treatment of diseases by targeting the microbiome have been widely investigated, and some therapies have been successfully applied in the clinic. The administration of probiotics is reported to help restore the health of patients more quickly. [14]

Fecal microbiome transplantation has exhibited better clinical efficacy than antibiotics in the treatment of *C. difficile* infections**.[15]** Substantial progress has also been made in the treatment of liver diseases by modulating the gut microbiome. A clinical trial showed that probiotic reduces liver disease severity and hospitalization in patients with cirrhosis [16]; the administration of *Lactobacillus salivarius* or *Pediococcuspentosaceus* improves the acute liver injury induced by D-galactosamine in rats **[1**7]. Furthermore, the regulation of the human microbiome plays important roles in the treatment of GI diseases, such as infectious diarrhea, antibiotic-associated diarrhea, inflammatory bowel disease, and necrotizing enterocolitis. For example, the oral administration of a mixture of 17 Clostridia strains from the human microbiota to adult mice was found to attenuate disease in models of colitis and allergic diarrhea.[18] Modulation of the gut microbiome may also contribute to the treatment of cancer. Iida et al. [19] reported that optimal responses to cancer therapy require an intact commensal microbiota that mediates the therapy effects by modulating myeloid-derived cell functions in the tumor microenvironment. Viaud *et al.* [20] reported that the gut microbiota helps to shape the anticancer immune response of cyclophosphamide. In addition,

many clinical studies have shown that probiotics and their products have outstanding effects on the treatment of allergic diseases, especially those in infants [21]

Chronic diseases such as obesity, inflammatory bowel disease (IBD), diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma have been associated with the human microbiota. If we have more emphasizes on nutrition, diet modification and restoring microbiota could prevent the occurrence of chronic diseases.

The symbiotic (Prebiotics and probiotics) and tryptophan rich diet could be beneficial for restoring of microbiota and also helpful in the absorption and metabolism of tryptophan may be helpful for prevention and treatment of various chronic diseases and it also overcome the adverse effect of medication which is used for the treatment of such diseases.

ENCLOSURE-II

2.REVIEW OF LITERATURE

Broekaert, Ilse J. MD et al. They have reviewed that as a probiotics as and nutrition in diet definitely normalize the microbiota and they have demonstrated a crucial role of nutrition in the prevention of chronic disease. As per their views the positive, strain specific effects of probiotics have been shown in diarrheal disease, inflammatory bowel diseases, irritable bowel syndrome , H. Pylori induced gastritis and in the prevention of cancer. According to him probiotics act as an adjuvant in the prevention and treatment of a wide variety of chronic diseases[22]

Broekaert, Ilse J. MD et al. studied Bifidobacterium (eg, B. longum, B. breve , and B. infantis) and Lactobacillus (eg, L. helveticus , L. rhamnosus), with doses between 109 and 1010 colonyforming units for 2 weeks in animals and 4 weeks in humans. These probiotics showed efficacy in improving psychiatric disorder related behaviors including anxiety, depression, autism spectrum disorder (ASD), obsessive-compulsive disorder, and memory abilities, including spatial and non-spatial memory. [23]

Huiying Wang, In-Seon Lee et al. they have studied higher intakes of dietary fiber are linked to less cardiovascular disease and fiber plays a role in gut health. Inulin, fructo-oligosaccharides, and other oligosaccharides are the best well known prebiotics which stimulate Bifidobacterium, a beneficial bacterial genus have many important function in gut.[24]

Joanne Slavin et al. Authors have reviewed the prebiotic intake may reduce the prevalence and duration of infectious and antibiotic associated diarrhea, inflammatory bowel disease, cardiovascular disease and obesity. Also studied its protective effect to prevent colon cancer and it enhances the bioavailability and uptake of minerals including calcium, magnesium and iron. [25]

Linda S. Zhang et al. They have studied tryptophan is an essential amino acids found in a variety of foods such as red meat, fish and eggs. The catabolism of amino acids by the microbiota (Lactobacillus spp., Clostridium sporogenes) into metabolites IPA which is a pregnane X receptor (PXR) agonist, particularly in the presence of indole. A wide-range of PXR agonists inhibit NF-κB and downregulation of intestinal tumor necrosis factor (TNF)-α and up regulation of junction proteins by IPA requires PXR. IPA also potently scavenges hydroxyl radicals, thereby protecting against oxidative injury in various animal models. [26]

Joanne Slavin et al. They have studied in brief the role of L-tryptophan as precursors for the biosynthesis of neurotransmitter and hormone including melatonin. The metabolism and absorption is enhanced in presence of gut flora.[25]

Tor C. Savidge et al. They have studied diet, probiotics and antibiotic use may regulate enteric neurotransmission through modulation of microbial communities. A particular emphasis is placed on bacterial metabolite regulation of enteric nervous system function in the intestine.[27]

Paul et al. They have studied indigeneious microbiota produce low molecular weight substances that potentially interact with the tissue cellular environment to modulate signaling pathways and regulate gene expression. Recently, these low molecular weight substances have been shown to influence epigenetic modifications, chromatin remodeling and other signaling molecules, ultimately regulating apoptosis, cellular differentiation, and inflammation. Therefore, the epigenetic changes brought about by microbiota may contribute to prevention or therapeutic intervention of cancer or other diseases.[28]

ENCLOSURE-III

3. NEED FOR THE STUDY

Metabolic disorders, including type 2 diabetes (T2DM), cardiovascular disease (CVD) and various chronic diseases present an increasing public health concern and can significantly undermine an individual's quality of life. The relative risk of CVD, the primary cause of death in T2DM patients, is two to four times higher in people with T2DM compared with those who are non-diabetic. The prevalence of metabolic disorders has been associated with dynamic changes in dietary macronutrient intake and lifestyle changes over recent decades. Recently, the scientific community has considered alteration in gut microbiota composition to constitute one of the most probable factors in the development of metabolic disorders. The altered gut microbiota composition is strongly conducive to increased adiposity, cell dysfunction, metabolic endotoxemia, systemic inflammation, and oxidative stress. Probiotics and prebiotics can ameliorate T2DM and CVD, and inflammatory through improvement of gut microbiota, which in turn leads to insulin-signaling stimulation and cholesterol-lowering effects. We analyze the currently available data to ascertain further potential benefits and limitations of probiotics and prebiotics and tryptophan rich diet in the treatment of metabolic disorders, including T2DM, CVD, and other disease (obesity).

For the treatment of various chronic diseases available medicines have severe adverse effect and they alter the normal physiology by economically as well as physically for to avoid this alternative approaches for the prevention of chronic diseases with symbiotic and diet modification could be possible.

ENCLOSURE -IV

4. AIM AND OBJECTIVES:

Objectives:

1. To design and evaluation of dose and route of symbiotic.
2. Development of Model for management of chronic diseases
3. To evaluate prophylaxis ability on chronic disease by using animal models
4. The other major objective is to rationalize the information by statistically proving its effects pre-clinically.

ENCLOSURE-V

5. MATERIALS AND METHODS OF RESEARCH

Following are the few models for Chronic Diseases:

5.1 Collection of prebiotic, probiotic and tryptophan from market.

5.2 Evaluations of antidepressant activity: The combination of symbiotic and tryptophan will be subjected for study the antidepressant activity using screening model.

5.3 Evaluations of antidiabetic activity: The combination of symbiotic and tryptophan will be subjected for study the antidiabetic activity using screening model.

5.4 Source of data: Source and Collection of data: The data is generated using laboratory experimental techniques. Albino rats and mice will be used for this purpose. Data will be acquired from national and international journals and also from other reputed libraries. The day to day development will be updated by conducting literature survey through, journal, magazine and online sources.

Animals:

Wistar rats weighing 150-250g will be used. The animals will be maintained under controlled conditions of temperature (23 ± 2º C) and 12-h light-dark cycles. The animals will be randomized into experimental and control groups and housed each in two sanitized polypropylene cages containing sterile paddy husk as bedding. They will have free access to standard pellets as basal diet and water *ad libitum.*

ENCLOSURE-VI

Data and statistical analysis:

Data were expressed as mean ± standard error of mean (SEM). Significance between two groups was determined using the unpaired student's ttest. Statistical comparisons between all groups were performed by using two tailedoneway ANOVA followed by the Dunnett test. P-values <0.05 were considered statistically significant.

6. PLAN OF WORK

a. Literature survey

b. Collection of symbiotic and tryptophan

c. Pharmacological evaluations

Evaluations of antidepressant activity

Evaluations of anti-obesity activity

Evaluation of antidiabetic activity

Evaluations of inflammatory bowel disease activity

d. Statistical interpretation

7. DURATION OF WORK:

Sr. No.	Activity	Tentative Duration (months)	Tentative Duration (Years)
1.	Literature Review	06	01
2.	Collection of prebiotic, probiotic and tryptophan from market and dose finalization	03	
3.	Designing of models for some chronic diseases	03	
4.	Evaluations of antidepressant activity	02	01
5.	Evaluations of anti-obesity activity	05	
6.	Evaluations of ant diabetic activity	05	
7.	Evaluations of inflammatory bowel disease activity	06	01
8.	Compilation, interpretation of data and writing of thesis	06	
7.	Total	36	03

8. RESEARCH OUTCOMES:

It has become clear in recent years that the human intestinal microbiota plays an important role in maintaining health and thus is an attractive target for clinical interventions. Scientists and clinicians have become increasingly interested in assessing the ability of probiotics and prebiotics to enhance the nutritional status of malnourished children, pregnant women, the elderly, and individuals with non-communicable disease-associated malnutrition. Probiotics are live microorganisms that are intended to have health benefits.. Although people often think of bacteria and other microorganisms as harmful "germs," many microorganisms help our bodies function properly. For example, bacteria that are normally present in our intestines help digest food, destroy disease-causing microorganisms, and produce vitamins. Large numbers of microorganisms live on and in our bodies. Many of the microorganisms in probiotic products are the same as or similar to microorganisms that naturally live in our bodies.

On the basis of scientific data and current evidence present study taken into consideration for to prevent the chronic disease with symbiotic and tryptophan rich diet.

ENCLOSURE –VII

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